Patent Application of

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for

COMPOSITIONS AND METHODS FOR TREATING LUNG CANCERS

BACKGROUND - SUMMARY

The current invention proposes administration, by inhalation, of therapeutically effective doses of certain sesquiterpene epoxides (trichothecenes), to inhibit proliferation of, or reduce viability of, neoplasms in the lungs. Methods are also provided for chemical debridement of diseased cells in the lungs of COPD patients as well as inhibition of inflammatory responses in the lungs, by inhalation of therapeutically effective doses of trichothecenes.

BACKGROUND

The most fundamental function a cell is protein synthesis (i.e. expression of its DNA). Proteins make up ~ 60% of a dry cell's mass by weight. In very broad and general terms, as cells mature and differentiate in the body, they reach an equilibrium in protein synthesis and protein degradation and settle down to perform their given function in this relative state of homeostasis. There are two notable exceptions that cause massive perturbations to this homeostasis: 1) when a cell is called upon to grow and divide and 2) when certain secretory cells are called upon to produce large

amounts of proteins for secretion. Although the cell signaling signaling pathways, intracellular transduction pathways, and spectrum of protein(s) to be produced are quite different in growth versus secretion, normal growth and secretion events share one major similarity in their end result: massively accelerated protein synthesis. A cell that is called on to grow (cycling cell) has as much as 5 times the protein synthesis activity of a non cycling cell. Likewise, secretory cells such as those of the immune system become protein factories producing massive amounts of antibodies, mediators, growth factors, or other proteins when stimulated to do so.

There are also abnormal conditions such as cancer and viral infections that share the same property of hyperactive protein synthesis versus normal quiescent cells. Cancer is a growth and divide type event, and even though the signaling mechanism is different in that it is self-induced intracellularly by several genetic mutations, the end result is also hyperaccelerated protein synthesis characteristic of a cycling cell. Viruses invade a cell, parasitize the host cellular machinery, and convert the cell into a factory producing massive amounts of viral proteins, much like a secretory cell.

Inhibiting protein synthesis affects cells in a dose dependent manner and affects actively cycling cells differently than non cycling cells. At low doses, protein synthesis inhibitors (PSIs) stop actively cycling cells from cycling without killing them (hereinafter referred to as inhibitory or G zero inducing dose) . Inhibitory doses also stop hyperaccelerated protein synthesis by secretory cells. At moderate doses PSIs exhibit toxicity to actively cycling cells (hereinafter referred to as the cytotoxic dose). At high doses, PSIs exhibit toxicity to all cells (hereinafter referred to as the toxic dose).

Prior art has recognized the potential of using protein synthesis inhibitors as chemotherapeutics (cytotoxic doses). However, prior art was unable to overcome hematologic toxicity issues (as bone marrow cells are also actively cycling and subject to the cytotoxicity) related to using PSIs and the area was largely abandoned. There are no chemotherapeutics in use today that function by broad spectrum protein synthesis inhibition. A brief background of prior art chemotherapy and chemotherapeutic administration methods is summarized below as it is relevant to several novel methods of present invention.

Chemotherapeutics (per HPIM)

Most chemotherapeutic agents in use today are cell cycle active; that is, they are cytotoxic mainly to actively cycling cells. Alkylating agents are among the most widely used anti tumor agents and are efficient at cross-linking DNA, leading to strand breakage. Alkylating agents include cyclophosphamide, ifosfamide, melphalan, busulfan, mechlorethamine (nitrogen mustard), chlorambucil, thiotepa, carmustine, lomustine as well as platinum compounds such as cisplatin and carboplatin, which are not true alkylating agents also lead to covalent cross linking of DNA. Purine/pyrimidine analogs/antimetabolites induce cytotoxicity by serving as false substrates in biochemical pathways. They include cytarabine, fluorouracil, gemcitabine, cladribine, fludarabine, pentostatin, hydroxyurea, and methotrexate. Topoisomerase inhibitors interfere with the enzymes topoisomerase 1 and topoisomerase 2, responsible for mediating conformational and topological changes in the DNA required during transcription and replication. These agents include daunorubicin, doxorubicin, idarubicin, etoposide, teniposide, dactinomycin, and mitoxantrone. Plant Alkaloids include vincristine, vinblastine, and vinorelbine which inhibit microtubule assembly by binding to tubulin and docetaxel and paclitaxel which function by stabilizing microtubules and preventing their disassembly. Antitumor Antibiotics include bleomycin that induces DNA strand breakage through free radical generation and Mitomycin C which cross links DNA. Other Agents include dacarbazine and procarbazine which act as alkylating agents to damage DNA and L-Asparaginase, the only enzyme used as a anti tumor agent, which acts by depletion of extracellular pools of asparagine.

Since most chemotherapeutics work by damaging DNA they are both mutagenic and carcinogenic. PSIs are cell cycle active but not mutagenic or carcinogenic.

Principles of Chemotherapeutic Administration

Chemotherapeutic agents exhibit a dose response effect. At sufficiently low concentrations no cytotoxicity is observed. At increasing concentrations, cell kill is proportional to drug exposure. At high concentrations, the effect reaches a plateau. Drugs that are cell cycle active, but not phase specific, characteristically have steep dose response curves: An increase in the drug concentration by an order of magnitude or more results in a proportional increase in tumor cell kill. By contrast,

the dose response curve of phase specific agents typically is linear over only a narrow range. These agents are less suitable for dose escalation and increased tumor cell kill is observed after prolonged exposure as a larger percentage of the tumor cells enter the cell cycle.

Chemotherapy employs two principles in administration: Therapeutic Index Dosaging and Cyclical Administration (HPIM 527 -528 Pharmocodynamics section).

The therapeutic index represents the difference between the response of the tumor and response of normal tissue for a given dose of chemotherapeutic. Normal cells are also susceptible to the cytotoxic effects of chemotherapeutic drugs and exhibit a dose-response effect, but the response curve is shifted relative to that of malignant cells (see HPIM P. 528, figure 86-3). This difference represents the therapeutic index. The toxicity to normal tissue that limits further dose escalation is the "dose-limiting toxicity". The dose just below this point is the "maximum tolerated dose". Proliferative normal tissues such as the bone marrow and gastrointestinal mucosa are generally the most susceptible to chemotherapy-induced toxicity. The usefulness of many chemotherapeutics is limited by the fact that they have a narrow therapeutic index.

Cyclical administration is used to allow normal rapidly proliferating tissue such as hemopoietic stem cells to recover and blood counts to reach their normal levels. Most chemotherapeutics are administered in cycles of 21 to 28 days and 6 to 8 cycles are typically used. The number of administration cycles required to completely eradicate a tumor is dependent on the tumor kill rate of the therapeutic the number of cycles required is determined using the Skipper log cell kill model. To completely eradicate a tumor it is necessary to get below the mathematical 1 surviving cell number. As an example, to kill a 10 billion cell tumor with a chemotherapeutic that kills 95% of the tumor cells each administration cycle (5% survive) would require 8 cycles of chemotherapy (i.e. 10,000,000,000 x .05 x .05 x.05 x .05 x .05 x.05 x .05 x .05 = .39). However, this assumes the tumor does not grow in the period when the chemotherapeutic is not administered ("off" period).

Prior Art Attempts to Use PSIs as Chemotherapeutics

Prior art's attempts to use PSIs as chemotherapeutics have failed. U.S. Patents 4,744,981 and 4,906,452 establish prior art utility of using trichothecenes as chemotherapeutics against cancer as well as the abject failure of administering Anguidine by injection because of "considerable hematologic toxicity in the Phase II trial". Prior art then attempted to obviate systemic cytotoxicity issues by replacing the function of the macrocyclic ring (responsible for non-specific cellular internalization) with monoclonal antibodies to deliver the toxins to target cell populations (U.S. Pat. 4,906,452 and 4,744,981). Prior art also pursued glycosylation to increase the solubility of trichothecenes in the blood.

The present invention takes the novel, unobvious, and exactly opposite approach to resolve the systemic cytotoxicity issue. Current invention reverses the direction of administration over prior art, going from tissue to blood versus from blood to tissue. Current invention embraces the lack of solubility in blood (exactly opposite to prior art glycosylation) to keep therapeutics of present invention out of general circulation. Current invention also embraces the use of non specific cellular internalization (exactly opposite from prior art), using this attribute to rapidly localize trichothecenes to the intra-organ, intercellular gap junction transport system to prevent distant lymphatic or circulatory transport and systemic cytotoxicity. Using principles of therapeutic index dosaging, the current invention uses normal lung tissue itself to "filter out", retain, and eventually inactivate the trichothecene, preventing appreciable systemic cytotoxicity as well as providing prophylactic activity against development of neoplasms in the lungs.

SUMMARY OF THE INVENTION

The present invention both overcomes the inability of prior art to use PSIs as chemotherapeutics and present invention also provides several other novel therapeutic treatment methods not envisioned or anticipated under prior art.

First, present invention will provide methods for using PSIs as chemotherapeutics. It will be shown how certain trichothecenes can be used to provide superior chemotherapy for treatment of cancers in the lung, with a further advantage of achieving specificity to the cancer tissues in the lungs without toxicity to normal lung tissue and without systemic cytotoxicity to other normal rapidly proliferating cells. This is a goal that has eluded prior art.

Second, present invention will provide novel and unobvious methods for indefinitely inhibiting growth of cancers and cancer related angiogenesis in the lungs through non toxic doses of therapeutics of present invention. This has not been envisioned under prior art and as such has no true comparable in prior art.

Third, present invention will provide novel methods of enhancing existing chemotherapeutic treatments of cancers in the lungs. Present invention will provide methods for increasing the integrity of the Skipper log cell kill model as well as providing methods for reducing the ability of multi drug resistant cancers to fight off chemotherapeutics.

Fourth, present invention will provide novel methods of treating patients suffering from Chronic Obstructive Pulmonary Disease (COPD) through therapeutics of present invention. Present invention will provide methods of chemical debridement of malfunctioning lung cells in COPD patients as well as methods for management of any potential subsequent inflammatory response.

Fifth, present invention will provide novel methods of inhibiting inflammation in the lungs by inhibiting or preventing synthesis of protein mediators of anaphylaxis.

BRIEF DESCRIPTION OF DRAWING FIGURES

FIG. 1A shows the hyperactive protein synthesis inhibiting dose profile in human cells of Roridin A, a representative macrocyclic trichothecene.

FIG. 1B shows the hyperactive protein synthesis inhibiting dose profile in human cells of Satratoxin G, a representative macrocyclic trichothecene.

FIG. 2A shows the hyperactive protein synthesis inhibiting dose profile in human cells of T - 2, a representative simple trichothecene.

FIG. 2B shows the hyperactive protein synthesis inhibiting dose profile in human cells of DAS, a representative simple trichothecene.

FIG. 3 shows the hyperactive Cyclin/CDK protein synthesis activity required at various points in the cell cycle to drive the cell through the cell cycle (i.e. the cell cycle control system).

FIG. 4 shows the molecular pathways depending on the hyperactive protein synthesis of the G1 cyclin/CDK proteins required to drive the cell through the G1 phase of the cell cycle.

DETAILED DESCRIPTION OF THE INVENTION

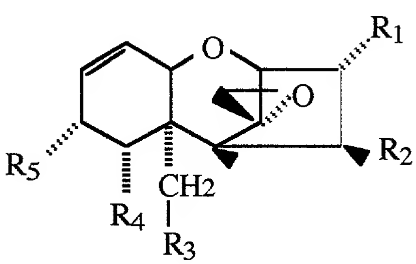
The treatments disclosed below involve administration of biologically active trichothecenes by inhalation to kill cancer cell populations in the lungs, to inhibit the growth of cancer and endothelial cell populations in the lungs, to destroy diseased tissue in the lungs, and to inhibit production of mediators of anaphylaxis. Materials and methods for achieving this are described below.

<u>Trichothecenes Defined</u>

Fungi of the genera Fusarium, Myrotecium, Trichoderma, Stachybotrys and others produce Trichothecene mycotoxins. Trichothecenes constitute a family of fungal sesquiterpene epoxides that inhibit protein synthesis. Trichothecene mycotoxins are low molecular weight (250-700 daltons), non volatile compounds, and of over 150 trichothecenes have been identified. There are two broad classes: those that have only a central sesquiterpenoid structure and those that have an additional macrocyclic ring (simple and macrocyclic trichothecenes, respectively).

As used in this application, “therapeutics”, “biologically active agent”, or “trichothecene” are defined as either simple or macrocyclic trichothecenes and include molecules of the following general chemical formulas: Simple trichothecenes are categorized into three groups with the following chemical formulas:

<u>Group A:</u>



Wherein R_1 is H, OH, or $O-\overset{\overset{O}{\|}}{C}-CH_3$;

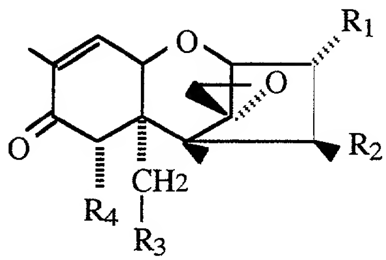
R_2 is H, OH, or $O-\overset{\overset{O}{\|}}{C}-CH_3$;

R_3 is H, OH, or $O-C-CH_3$;

R_4 is H or OH; and

R_5 is H, OH, $O-\overset{\overset{O}{\|}}{C}-CH_3$ or $\overset{\overset{O}{\|}}{C}-O-CH_2(CH_3)_2$.

Group B:



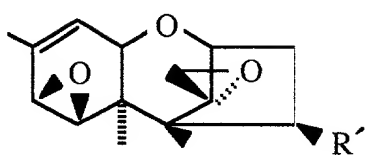
Wherein R_1 is H, OH, or $O-\overset{\overset{O}{\|}}{C}-CH_3$;

R_2 is H, OH, $O-\overset{\overset{O}{\|}}{C}-CH_3$ or $O-\overset{\overset{O}{\|}}{C}-CH{=}CH-CH_3$;

R_3 is H, OH, or $O-\overset{\overset{O}{\|}}{C}-CH_3$;

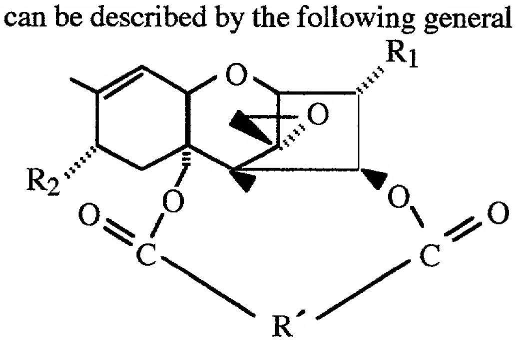
R_4 is H, OH, or $O-\overset{\overset{O}{\|}}{C}-CH_3$;

Group C:



Wherein R´ is OH or $O-\overset{\overset{O}{\|}}{C}-CH{=}CH-CH_3$.

Macrocyclic Trichothecenes can be described by the following general chemical formulas:



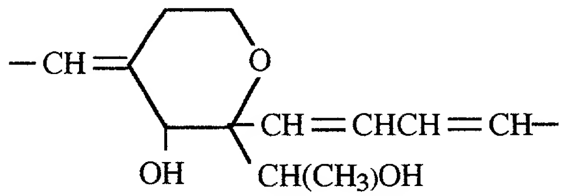
Wherein R_1 is OH, or $O\overset{\overset{O}{\|}}{C}-CH_3$;

R_2 is H, OH,$O-\overset{\overset{O}{\|}}{C}-CH3$ or $OCOCH_2CH(CH_3)_2$; and

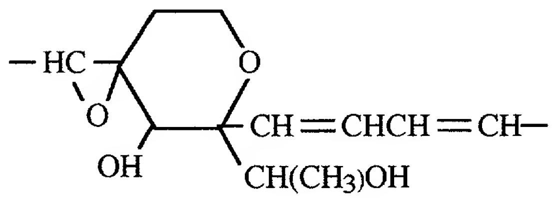
R´ is any molecule composed predominantly, or in its entirety, of combinations of C, H, and O:

Some representative examples of R′ include:

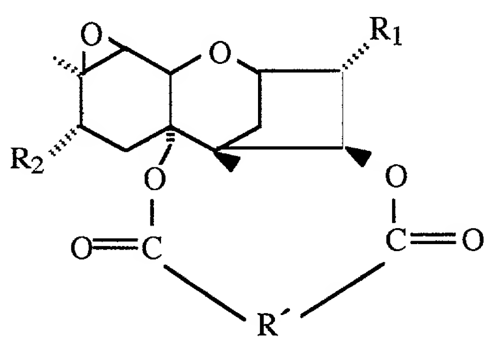
Satratoxin H:



Satratoxin G:



or molecules of the following general formula:



Wherein R_1 is H, OH, or $O{-}C({=}O){-}CH_3$;

R_2 is H, OH, $O{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}CH_3$ or $OCOCH_2CH(CH_3)_2$; and

R′ is any molecule composed predominantly, or in its entirety, of combinations of C, H, and O.

A more comprehensive listing of trichothecenes is included in U.S. Patents 4,744,981 and 4,906,452 , incorporated herein by reference.

Trichothecenes are fast acting potent inhibitors of protein synthesis in eucaryotic cells. Their main effects are on rapidly proliferating tissues such as bone marrow, skin, mucosa epithelia, and germ

cells. The sesquiterpenoid ring binds to ribosomes, inhibiting protein synthesis. The macrocyclic ring enhances cell binding and internalization.

Trichothecenes are invisible to the immune system since they neither contain nor produce amino acids. Since trichothecene molecules contain only carbon, hydrogen, and oxygen they are not subject to proteolytic degradation. U.S Pat. No. 4,906,452 (column 11 first paragraph) further discloses that some studies of the rates at which certain trichothecenes are converted into biologically inactive molecules (apotrichothecenes) found that macrocyclic trichothecenes are inactivated quite slowly and only by intracellular acid catalysis as might occur in lyzosomes.

Trichothecenes are extremely stable to heat and ultraviolet light inactivation. Heating to 500° F for 30 minutes is required for inactivation. Brief exposure to NaOH destroys toxic activity. These substances are relatively insoluble in water but are highly soluble in ethanol, methanol, and propylene glycol.

<u>Preparation of Trichothecenes</u>

Fungi can be grown in culture and the trichothecenes extracted by centrifugal partition chromatography as described in Tani et. al. and described in other literature such as Onji et. al. *(Onji, Y., Aoki, Y., Yamazoe, Y., Dohi, Y., and Moriyamam, T., 1988 Isolation of nivalenol and fusarenon-X from pressed barley culture by centrifugal partition chromatography, Journal Liquid Chromatography, 11:2537-2546)* or Jarvis et al. *(Jarvis, B.B., R.M. Eppley, and E.P. Mazzola, 1983 Chemistry and Bioproduction of the Macrocyclic Trichothecenes, p 20-38. In Y. Ueno, Trichothecenes: chemical, biological, and toxicological aspects, vol 4. Elsevier Science Publishing Inc., New York)* or Sorensen et al. *(Sorenson, W.G., Frazer, D. G., Jarvis, B.B., Simpson, J., and Robinson, V.A., Trichothecene Mycotoxins in Aerosolized Conidia of Stachybotrys atra, June 1987 Applied and Environmental Microbiology, Vol. 53 No. 6, p. 1370-1375)* where S. atra was grown on sterile rice, autoclaved, dried, and then aerosolized by acoustic vibration and collected on glass-fiber filters and extracted with 90% aqueous methanol.

Alternatively, certain trichothecene mycotoxins can be purchased from companies such as Sigma

Chemical Co. St. Louis MO, USA or Wako Pure Chemical Industries, Ltd., Japan, or Wellcome Research Labs, Buckinghamshire, England or Boehringer-Mannheim, Manheim, West Germany .

The preferred embodiment of current invention envisions using Satratoxin H as well as other combinations of trichothecenes such as satratoxins G, H, F, roridin E, verrucarin J, and trichoverrols A and B for reasons discussed later. These trichothecenes can be obtained by growing the fungus stachybotrys atra on sterile rice and extracting the trichothecenes by centrifugal partition chromatography as described in Tani et. al. or having it grown on sterile rice, autoclaved, dried, and then aerosolized by acoustic vibration and collected on glass-fiber filters and extracted with 90% aqueous methanol as described by Sorensen et al..

Method of Administration

Preferred embodiment of current invention administers trichothecenes by inhalation in their raw dry powder form through commercially available dry powder inhaler devices such as the Pulmicort Turbuhaler breath activated dry powder inhaler (Astra USA Inc., Westborough, MA) or Galaxo Wellcome's Diskus inhaler. However, any suitable commercially available inhaler devices, nebulizers, or any other suitable means and in combination with any suitable solution or device to facilitate inhalation, retention, or absorption by the lungs may be used. Such devices are commercially available from sources such as Self Care, Emeryville, CA, USA and enclosed examples include the Lumiscope Ultrasonic Nebulizer, the Dura-Neb® 3000 Portable compressor driven nebulizer, the PARI LC plus Nebulizer, the Omron CompAir Compressor Nebulizer System, the SpaceChamber™ aerosol spacer, and other devices.

Alternatively, therapeutics of present invention may also be administered by cigarette (either tobacco or other). Trichothecenes require temperatures of 500° F for 30 minutes for inactivation allowing them to still be biologically active after being inhaled through a lit cigarette. The diseases considered for treatment under present invention such as lung cancer and COPD are primarily caused by smoking. A large number of patients do not quit smoking even after acquiring lung cancer. From a purely medical standpoint, cessation of cigarette smoking does not markedly decrease the cured cancer patient's risk of second malignancy (HPIM p. 501). Methods of mixing

additives with tobacco are established in prior art in the tobacco industry. Measurement of effective dosages of trichothecenes inhaled per cigarette can be determined by using equipment developed in prior art for testing tar and nicotine content inhaled per cigarette or using glass fiber filters and pumps (emulating human inhalation patterns from cigarettes) and measurement of collected dosages could be performed after extraction with 90% aqueous methanol as described in Sorensen et. al. The appropriate number of cigarettes to be smoked to deliver the desired therapeutically effective dosages would then be determined.

<u>Dose Determination</u>

Fig. 1A and 1B show the hyperactive protein synthesis inhibiting dose profile of roridin A and satratoxin G, respectively. Both roridin A and satratoxin G are macrocyclic trichothecenes. By ~ 5 ng/ml both had inhibited almost 100% of the hyperactive protein synthesis. Both did not reduce cell viability at concentrations of 10 ng/ml or less.

Figure 2A and 2B show the hyperactive protein synthesis inhibiting dose profile of T-2 and DAS, respectively. Both T-2 and DAS are simple trichothecenes. By doses of 5 ng/ml both had inhibited ~ 99% of hyperactive protein synthesis. Neither reduced cell viability at concentrations up to 200 ng/ml.

The hyperactive protein synthesis inhibiting profiles were constructed from data collected from in vitro experiments using human epidermoid cells, virally infected with HSV-2 to induce a hyperactive state of protein synthesis, and conducted and reported by Okazaki et. al. in the attached Journal of Agricultural and Biological Chemistry articles.

Conversion of in vitro concentrations to dosages required to achieve in vivo concentrations would be performed by simple mathematical methods. As an example, if a patients has an average lung weight of ~350 grams and one desires to achieve a 5 ng/ml concentration of Satratoxin one would need to administer ~ 1,728 ng of dry satratoxin (i.e. 1 gram = .9875 ml, 350 gram lungs ~ 346 ml, 346 ml X 5 ng/ml = 1728 ng.) by inhalation methods described above. Adjustments would be made for individual lung size differences and additional tumor mass where applicable.

Inhibitory, Cytotoxic, and Toxic doses are used in various treatment applications discussed later in the application. Using the data from the viral protein synthesis inhibiting model referenced above, dose guidelines for use in the Inhibitory, Cytotoxic, and Toxic embodiments of present invention may look roughly as follows:

TABLE 1: Trichothecene in Vitro Concentrations (ng/ml)

	Inhibitory				Cytotoxic		Toxic		Lethal
	50%	80%	90%	100%	Lo	Hi	Lo	Hi	50%
Roridin A	1.4	2.0	3.3	5.0	6	10	11	?	?
Satratoxin	1.5	2.4	3.9	5.0	6	10	11	?	?
T-2	1.6	3.5	4.3	5.0	6	200	?	?	1,210,000*
DAS	2.3	4.0	4.5	5.0	6	200	?	?	?

TABLE 2: In Vivo Dose for Average 350 Gram Lungs (ng)

	Inhibitory				Cytotoxic		Toxic		Lethal
	50%	80%	90%	100%	Lo	Hi	Lo	Hi	50%
Roridin A	484	691	1141	1728	2074	3456	3802	?	?
Satratoxin	518	830	1348	1728	2074	3456	3802	?	?
T-2	553	1210	1486	1728	2074	69125	?	?	84,700,000*
DAS	795	1383	1555	1728	2074	69125	?	?	

** The T-2 lethal dose by inhalation for 50% mortality in humans was obtained from an AMRIID study, discussed later in the application.*

The above are ballpark numbers for illustrative purposes and precise levels need to be determined by more in vitro testing in other models and on other cell lines as well as further refined under prior art protocols for Phase I & II clinical trials.

Alternative Method of Therapeutic Dose Determination

Although specific dose profiles for therapeutics have been discussed above, present invention can easily be extended to using various other trichothecenes as well as combinations of trichothecenes (to affect the depth of penetration of therapeutics of present invention). Accordingly, a general method for dosage determination of other trichothecenes is provided below.

Human cell lines, including human lung cell lines, are commercially available from several sources including ATCC - American Type Culture Collection, Manassas, VA, USA or ECACC - European Collection of Cell Cultures, Salisbury, Wiltshire, UK or DSMZ - German Collection of Microorganisms & Cell Cultures, Braunschweig, Germany or IZSBS - Istituto Zooprofilattico Sperimentale, Brescia, Italy or ICLC - Interlab Cell Line Collection, Genova, Italy or ECBR - European Collection for Biomedical Research, Genova, Italy or any other suitable supplier. Human lung cell lines available include both normal and malignant cell lines. As an example, LL24 is a human lung cell line available from ECACC. Examples of malignant cell lines include A-427 human lung carcinoma available from DSMZ and COR-L23 human large cell lung carcinoma available from ECACC, however any other suitable cell line from any other suitable source may be used.

To establish inhibitory, cytotoxic, toxic, and lethal concentrations human lung cell lines (both normal and malignant) would be grown in culture and exposed to various concentrations of trichothecenes by methods described in Okazaki et al. or Tani et al. where human cell lines were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS). Trichothecenes would be dissolved in dimethyl sulfoxide at a concentration of 20 mg/ml and diluted in Eagle's MEM. Stock solutions (200 μg/ml) could be prepared, passed through a 450 - nm Millipore membrane filter and stored at - 20° C until use. Tissue culture plates would be seeded with normal human lung cell lines and other culture plates would be seeded with human lung cancer cell lines. Both sets of cells would then be allowed to proliferate at 37° C until confluent monolayers had formed. The culture plates would then be exposed to various concentrations of the trichothecenes and the number of viable cells periodically counted using trypan blue exclusion after trypsinization. A "Normal Tissue Response" and "Malignant Tissue Response" profile would then be constructed for various trichothecene concentrations under prior art methods as illustrated in HPIM Figure 86-3, pg. 528 as part of constructing a Therapeutic Index profile. Observed recovery time of the malignant cells (below cytotoxic dose levels) would serve as an indicator of intracellular inactivation time for purposes of determining periodicity of administration cycles (versus prior art which uses blood count recovery times to determine

periodicity between administration cycles).

National Cancer Institutes (NCI) protocols for animal and human testing would subsequently be performed to refine dosage administration. Currently, new anti tumor compounds are first tested against human cancer cell lines as described above. Agents demonstrating in vitro antitumor activity are then tested against a panel of human tumor xenografts in nude mice. Human clinical trials start with Phase I which is primarily concerned with dosage determination and safety before efficacy is tested in Phase II. Present invention envisions following conventional, prior art, NCI protocols.

Cytotoxic Activity and Safety by Inhalation

<u>The Cleveland Infant Model</u>

The cluster of infant deaths in Cleveland (NIEHS press release and MMWR report) demonstrated, in vivo, in humans, both a usable therapeutic index of certain trichothecenes at certain dosages when administered by inhalation, as well as their ability to localize in lung tissue without appreciably entering general circulation.

The Cleveland infants served as a model for rapidly proliferating tissues such as cancer. The mean age of the infants was ~ 10 weeks old (range 4 - 16 weeks). At this age, the lungs of infants are growing at an accelerated rate, similar to the accelerated growth rates of cancer. Likewise the effects of inhaled trichothecenes would be analogous to their effect on cancer.

The control group was represented by the adults living in the same household and inhaling the same trichothecenes. The median age of the infant's mothers was 20 years (range 15 - 29 years). At this age, the lungs of adults are not growing at an accelerated rate and are analogous to normal tissue as defined in the context of therapeutic index dose profile determination previously discussed.

Both groups were subjected (inadvertently) to airborne concentrations of trichothecenes produced

naturally by the fungus Stachybotrys atra. Trichothecenes produced by S. Atra include satratoxins H, G, F, roridin E, verrucarin J, and trichoverrols A and B.

The destruction of the rapidly proliferating tissues of the infants was so severe that it resulted in the death of at least 10 infants. No serious health problems were reported by the adults. This demonstrated that a useful therapeutic index exists at which tissue growing at an accelerated in the lungs is severely damaged or destroyed and normal tissue is not affected.

This also demonstrated the safety of using trichothecenes by inhalation in adults. Even in the infants, despite the acute pulmonary hemorrhage/hemosiderosis, the inhaled trichothecenes localized in the lungs and did not enter circulation where they would have caused systemic cytotoxicity. Laboratory findings on admission showed a normal white blood cell count (median = 13.8 cells/ cubic mm) in the infants. Red blood cell counts were consistent with the blood loss from the hemosiderosis. No other source of bleeding (i.e. gastrointestinal or nasopharyngeal) was identified during endoscopic evaluation.

The likely molecular basis for the "localization" of these trichothecenes is their ability to be rapidly internalized into cells because of their macrocyclic ring combined with their insolubility in blood, which would tend to keep them out of the circulatory system. The incredibly small size of trichothecenes (~ 1 nm or less) allows them to travel between cells (~ 2 - 4 nm spacing). Once internalized they can travel through gap junctions. Gap junctions allow molecules smaller than ~ 1000 daltons (~ 1.5 nm in diameter) to pass between connected cells and trichothecenes are comfortably under the size limitation at 250 - 700 daltons. Gap junction travel would tend to further localized trichothecenes within the organ or other connected tissue mass.

It is likely the inhaled trichothecenes are somewhat "trapped" between the lumen of the lungs on one side and the circulatory system on the other side, in which they are insoluble. In between this is the lung tissue in which they eventually internalize.

In therapeutically effective doses, trichothecenes are toxic to all rapidly proliferative cells, not just

cancer cells. Fortunately, the lungs do not normally contain rapidly proliferating cells.

Interference with protein synthesis has lethal consequences for actively cycling cells in various phases of the cell cycle. Actively cycling cells have as much as 500% more protein synthesis activity than non cycling cells. Several pathways are likely involved with the observed cytotoxicity of protein synthesis inhibitors to actively cycling cells. Temporary disruption of spindle microtubules preferentially kills many abnormally dividing cells. Microtubules are highly labile structures and are highly sensitive to concentrations of the protein tubulin for their rapid elongation and contraction. Interfering, even slightly, with the concentrations of free tubulin by a protein synthesis inhibitor, would prevent the critical concentration required for polymerization (rapid elongation) and formation of the mitotic spindle required for cell division. Reducing tubulin concentrations at an inappropriate time in the cell's division cycle would cause premature depolymerization (rapid shrinking) of the microtubules at an inappropriate time which would literally result in portions of the DNA being torn apart. Prior art chemotherapeutics that work by disruption of spindle microtubules to kill cancer include vinblastine, vincristine, paclitaxel and docetaxel. Vincristine and vinblastine inhibit microtubule assembly by binding to tubulin and thus are cytotoxic predominantly during the M phase of the cell cycle. Paclitaxel and docetaxel stabilize microtubules preventing their disassembly.

Several key proteins need to be switched on at a high rate of synthesis at various stages of the cell cycle. Histones, which are required for formation of new chromatin, are made at a high rate only in S phase and the same is true for some enzymes that manufacture deoxyribonucleotides and replicate DNA. In G1, large amounts of protein synthesis is required to grow the cell to nearly twice its size. Compositions of present invention interfere with all of these processes.

Although there have been studies on the rates at which trichothecenes are intracellularly converted into biologically inactive apotrichothecenes, the Cleveland infant model provides a rare glimpse of how slowly macrocyclic trichothecenes are inactivated, in vivo, in the lungs, after inhalation. All infants survived the first hospitalization and were discharged without evidence of hemoptysis after a median length of stay of 10 days, indicating an inactivation time in the ballpark of 10 days.

Lastly, the Cleveland infant model provides support that inhalable trichothecenes are uniquely suited for smoking related diseases. Environmental tobacco smoke was implicated in the severity of tissue destruction (NIEHS press release). Tobacco smoke is known to inactivate (and eventually destroy) cilia in the lungs. Cephalad movement of the mucus blanket at .5 - 1 mm/minute normally removes accumulated material from the lungs in ~24 hours. Inactivated or destroyed cilia would result in increased retention of trichothecenes in the lungs, further enhancing both efficacy and enhancing localization of the trichothecenes in the lungs. Consequently, smokers and smoke damaged lungs, which are the main target of therapeutics of present inventions, should benefit disproportionately over normal healthy lungs.

Prior art has failed to provide methods for using protein synthesis inhibitors as chemotherapeutics because of systemic toxicity. Present invention solves that problem for neoplasms of the lung by reversing direction of administration and using trichothecenes that, when administered by inhalation, localize and do not enter general circulation.

<u>Safety by Inhalation - AMRIID's Aerosolized Administration Model</u>

The safety of using trichothecenes by inhalation can be further substantiated by AMRIID's research on inhalation of aerosolized trichothecenes. The simple trichothecene T-2 was evaluated (see AMRIID Table 2). Even though trichothecenes are some of the most potent toxins by weight, when AMRIID administered T-2 in aerosolized form, T-2 came 25th out of 25 toxins tested for lethality by inhalation. AMRIID computed the LD50 (lethal dose to 50% of people) by inhalation as 1,210 μg/kg of body weight. This translates to a 84,700,000 ng dose of T-2 being inhaled by a 70 kg person to have a 50% chance of mortality. This contrasts with the 1728 ng maximum inhibitory dose (~ 49,000 X smaller) for T-2 proposed by present invention in the dose determination section of this application.

Inhibitory Activity

The bulk of this application deals with inhibitory, and not cytotoxic doses. This is fairly notable departure from prior art. Prior art administers cytotoxic doses of chemotherapeutics for short periods of time. By contrast, in one embodiment of present invention, trichothecenes are used as long term sustained treatment regimens more akin to a treatment of chronic lifelong diseases like diabetes rather than prior arts chemotherapeutic regimens. This embodiment address unmet needs such as holding the cancer and related angiogenesis in check indefinitely, in cases where neoplasms in the lung are unresponsive to chemotherapy. Another embodiment of present invention uses inhibitory doses administered during the time between administration cycles of prior art chemotherapeutics, to insure the integrity of the skipper log cell kill model. Another embodiment uses inhibitory doses for prophylactic purposes to prevent recurrence or metastasis of lung cancers. As such a brief discussion of the utility of trichothecenes as inhibitors follows.

Inhibiting the Cell Cycle Control System - Low doses of protein synthesis inhibitors stop actively cycling cells from cycling (without killing them). It has been shown that briefly inhibiting protein synthesis in early G1 (MBOC 896-897) stops cells from cycling by dismantling the cell cycle control system. After inactivation of the protein synthesis inhibitor, the cells require roughly 8 hours to rebuild their cell cycle control system and start cycling again.

A cycling cell is driven by an orderly sequence of events coordinated by the cell cycle control system. The cell cycle control system is dependent on hyperactive burst of protein synthesis of cyclins and cyclin dependent kinases (CDK). Fig. 3 shows the bursts of cyclin/CDK protein synthesis required to drive a cell through the various parts of the cell cycle. Fig. 4 shows the cell cycle control systems pathways involved in driving a cell through the G1 phase of the cell cycle. In a typical cell, inhibitory influences predominate and the normal state of a cell is not to cycle. In the non cycling cell, the Rb protein is bound to the E2F-DP1 transcription factor inhibiting it. When stimulated to proliferate, hyperactive protein synthesis of Cyclin/CDK is initiated, in particular Cyclin D/CDK4/6 complexes and Cyclin E/CDK2 complexes in G1, which phosphorylate the Rb protein, inactivating it, in turn activating the E2F-DP1 transcription factor.

Inhibiting hyperactive protein synthesis of cyclins and CDKs prevents a cell from cycling. Cyclins such as cyclin D have short half lives (~ 15 minutes for cyclin D) and inhibiting their synthesis reduces their concentrations rapidly. After inactivation of the protein synthesis inhibitor, the cell requires 8 or so hours to rebuild its cyclin/CDK levels before it can resume cycling again.

Inhibiting Overexpressed Oncogene Proteins - Cancer happens through mutations that cause over expression or inappropriate expression of proteins responsible for growth (oncogenes) or mutations that disable genes responsible for inhibiting growth (tumor-suppressor genes). In cancers whose genetic profile includes overexpression of certain oncogene proteins, compositions of present invention would provide an additional mechanism of action by directly inhibiting the hyperactive synthesis of these oncogene products. Coincidentally, lung cancer happens to have such a profile. Approximately 25% of all lung cancers are Small Cell Lung Cancer (SCLC) and 75 % of lung cancers are Non-small Cell Lung Cancer (NSCLC). Studies have shown that lung cancer cells have acquired a fairly large number of genetic lesions (perhaps 10 or more) and the profile of oncogene related protein product is heavily dominated by protein overexpression (HPIM pg 554) and as summarized below:

	Present in % of Cancer Cells Examined	
Oncogene Abnormalities	SCLC	NSCLC
myc family overexpression	> 50 %	> 50 %
bcl-2 overexpression	> 75 %	> 50 %
Her-2/neu overexpression	< 10 %	~30 %
Telomerase overexpression	> 90 %	> 90 %
ras mutations	< 1 %	~ 30 %

Trichothecenes, as a preferential inhibitor or hyperactive protein synthesis, would also inhibit overexpression of growth factor proteins coded for by these oncogenes.

Inhibition of Angiogenesis - Cancer also involves another process that is dependent on hyperactive

protein synthesis and hyperactive cell proliferation: angiogenesis. Angiogenesis is the growth of new blood vessels. Without angiogenesis a tumor cannot grow beyond the size of a pinhead (~ 1 to 2 cubic millimeters). The naturally occurring balance between stimulators and inhibitors of angiogenesis in the human body is also one in which inhibitory influences predominate. The rare instances in which angiogenesis occurs under normal conditions are wound healing, organ regeneration, embryonic development, and female reproductive processes. Also, when cells are deprived of oxygen or nutrients, they release angiogenic factors that induce new capillary growth which is why nearly all vertebrate cell are located within 50 μm of a capillary. Unnatural or disease conditions that involve angiogenesis include cancer. As tumor cells crowd each other, crowded tumor cells become deprived oxygen or nutrients, begin accelerated production of protein growth factors for angiogenesis such as VEGF, and issue these protein signals to the nearby endothelial cells (cells that form the wall of blood vessels). A normally dormant endothelial cell becomes activated, secretes enzymes that degrade the extracellular matrix (the surrounding tissue), invades the matrix, begins dividing and eventually the new endothelial cells organize into hollow tubes creating new networks of blood vessels. Endothelial cells can show a 100 fold increase in proliferation during neovascularization and it is known that tumors become vascularized at a size of less than 1250 μm^2.

Trichothecene administration would thus function two ways to inhibit angiogenesis. First, it would dismantle the cell cycle control system in endothelial cells by the same cyclin/CDK mechanisms described for cancer cells. Second, it would inhibit the excessive production of protein growth factors such as VEGF by tumor cells that initiate angiogenesis. In addition to acting as a mitogen, VEGF has been shown in vitro to downregulate integrins (cellular adhesion molecules) which in turn should release the endothelial cell from density dependent inhibition of cell division (via downregulation of proteins such as P27 shown in Fig. 4). Furthermore, the VEGF activated endothelial cell itself begins to secrete enzymes to degrade the extracellular matrix before it starts to divide. Although all of this is part of the normal sequence of the endothelial growth process, loosening cell to cell and cell to matrix adhesion, would likely provide the perfect situation where relatively untethered cancer cells could slip between relatively untethered endothelial cells and enter the blood stream. Trichothecene would inhibit VEGF production,

preventing downregulation of integrins and activation of endothelial cells, thus making it more difficult for cancer to enter into the bloodstream. Inhibiting angiogenesis not only starves the tumor of oxygen and nutrients but also reduces the probability of metastasis.

Interim Inhibition Applications - Inhibition of protein synthesis has an important application in the interim periods between chemotherapeutic administration cycles. Under prior art, the Skipper log cell kill model is predicated on cancer not growing at all in the interim periods between chemotherapeutic administration cycles. This is not likely, and if even one cell eventually survives because of this miscalculation, the cancer will recur. Administration of compositions of present invention can insure, with a much greater degree of certainty, that there will be no growth in the interim period, thus boosting the probability that every last cancer cell has been killed and the cancer will not recur.

Inhibition of MDR Proteins - Inhibition also has applications in the phenomenon of multidrug resistance (MDR). MDR is characterized by massively amplified numbers of copies of a small segment of the genome which often contains a specific gene, known as the multidrug resistance (mdrl) gene. This gene codes for a plasma-membrane-bound transport protein ATPase. ATPases are a diverse family of transport proteins and overexpression of MDR protein in human cancer cells enables them to pump drugs out of the cells. As protein synthesis inhibitors, composition of present invention would inhibit overexpression of MDR proteins, impairing the ability of cancer cells to pump out the chemotherapeutic(s). This would involve a rationally designed transition form discontinuation of administration of therapeutics of present invention and administration of chemotherapeutics to take advantage of the MDR impaired state.

Inhibiting cancer and related angiogenesis indefinitely, is a last hope for patients with neoplasms in the lungs that are inoperable and unresponsive to chemotherapy. A lifelong treatment regimen would generally be preferable to death and particularly when it may be as simple as periodically smoking a different type of cigarette.

The paradigm of present invention is also vastly different from prior art in that it is <u>organ specific</u>

rather than cancer specific. Both inhibitory and cytotoxic dose levels of compositions of present invention focus on clearing out or immediately arresting the growth of neoplasms in the lungs, no matter where they came from (e.g. whether indigenous or metastasized from the breast, prostate, melanoma etc...). Methods of present invention do not focus on eliminating lung cancers that have metastasized to distant sites. Methods of present invention will however provide another tool in the growing arsenal of tools for use against cancer.

Lastly, inhibitory methods of present invention provide a means for preventing or reducing inflammation in the lungs as well as the severity of allergy attacks and allergic asthma attacks. Allergens cause the conversion of B-cells into plasma cells, which in turn generate large quantities of immunoglobulins, however these cells are primarily in the bone marrow or blood and cannot be inhibited by therapeutics of present invention which are localized in the lungs. The target of these immunoglobulins, however, are mast cells which are located in the lungs and would be subject to inhibitory influences of trichothecenes. Mast cells are activated by the immunoglobulins and initiate hyperactive protein synthesis for newly formed mediators of anaphylaxis including cytokines, leukotrienes, thromboxane, and platelet activating factor. Activated mast cells also release preformed mediators of anaphylaxis including histamine, heparin, tryptase, kallikrein and chemotactic factors. Administration of inhibitory doses of trichothecenes well in advance of expected exposure to allergens would downregulate any of the preformed substances, muting any allergic response. Administering inhibitory doses at initiation of an allergic response would inhibit hyperactive production of the newly formed substances.

Prior art drug therapies include various strategies (HPIM 1423 -1425) for either interfering with the allergic response pathway or counteracting its symptoms. These include adrenergic stimulants, methylxanthines, anticholinergics, glucocorticoids, and mast cell-stabilizing agents. The mast stabilizing agents in use today, cromolyn and nedocromil, work by preventing the release of the preformed mediators of anaphylaxis. Compositions of present invention prevent synthesis of the newly formed mediators by the mast cells. Thus the novel mechanism of action of present invention would very well complement the prior art, providing a complete solutions to inhibition of release of preformed mediators (prior art) and inhibition of newly formed mediators (present

invention).

In cases such as COPD described below, CD8+ T lymphocytes and B lymphocytes comprise the primary inflammatory infiltrates in the lungs. If T or B lymphocytes are present in the lungs (other than in the blood), trichothecenes would provide a broad spectrum inflammatory inhibitor, interfering with multiple steps all along the inflammatory pathway, starting with inhibition of hyperactive production of effector proteins by T cells (e.g. IL-4, IL-13, IL-9), inhibition of hyperactive production of immunoglobulins (e.g. IgE) or other mediators by B cells, and inhibition of hyperactive synthesis of new mast cell product discussed above.

Toxic Activity

At high doses, trichothecenes are toxic to all cells, including normal, non proliferative cells. The present invention proposes a truly novel curative, rather than just palliative, solution for COPD based, in part, on the ability to inflict a certain percentage of destruction to non proliferative lung tissue and, in part, by using trichothecene's ability to suppress any potential inflammatory response that might otherwise result from having done so.

There are ~ 16 million COPD patients in the United States. COPD is a group of chronic, slowly progressive, respiratory disorders and is made up of emphysema and chronic bronchitis. COPD is the fourth leading cause of death and the only one of the top 10 for which mortality rates are still rising. COPD results from persistent inflammation (particularly from smoking) and results in narrowing of both large and small airways. Airway epithelium is characterized by squamous metaplasia (abnormal transformation of epithelium into scaly cells), atrophy of ciliated cells, an hypertrophy of mucus glands (increase in bulk). The remodeled epithelium actively produces cytokines that amplify and sustain the inflammatory process. Small airway transformation also includes overproduction of smooth muscle and goblet cells, peribronchial fibrosis, edema, intraluminal mucus plugs, and CD8+ T lymphocytes and B lymphocytes comprise the primary inflammatory infiltrates.

Palliative treatments include bronchodilators, glucocorticoids, and oxygen. Curative treatments are limited to lung transplants and Lung Volume Reduction Surgery (LVRS), an operation where severely emphysematous lung tissue is resected resulting in 25% to 50% improvement in airflow. LVRS mortality ranges from 5% to 18%, requires a hospital stay from 9 to 18 days, and costs $ 33,000 to $ 70,000 per case.

In one embodiment of present invention, trichothecenes are used in toxic doses to effectuate a respiratory tract chemexfoliation / chemical debridement treatment regimen somewhat akin to a "facial peel" for the lungs. Toxic doses would be administered by inhalation to kill off a desired amount of the remodeled, non-functional or mal functioning cells. Trichothecenes would also serve to inhibit any potential anaphylactic inflammatory response by mechanisms previously discussed for inhibition of local T, B, and mast cell proteins, however any of the prior art treatments for anaphylaxis could also be used. Since the main cause of COPD is smoking, administration by cigarette would provide smokers with a much more palatable treatment regimen than the alternative LVRS surgery.

METHODS OF USE OF COMPOSITIONS OF INVENTION

Examples are provided to further give guidance on methods of use of compositions of present invention as discussed above. Satratoxin H is used in the examples because it is known to be highly efficacious by inhalation in its raw, dry powder form (see U. of Minnesota printout enclosed) and the representative macrocyclics follow an almost identical dose curve so Satratoxin G's was used for H, however any suitable trichothecene could be substituted in the examples.

<u>Example 1: Use of Therapeutics as Stand Alone Treatment of Lung Cancer</u>

Lung cancer is the deadliest cancer resisting most conventional treatments. The average 5 year survival rate for all stages of lung cancer combined is currently only 14% . Chemotherapy today offers only "modest survival benefits (of 1 to 2 months)" and "complete clinical regression of tumor ... occurs in less than 5 % of cases" and as such use of prior art chemotherapy "requires

careful judgment to balance potential benefits and toxicity" (HPIM p 560). Clearly a better solution is needed over prior art.

Example 1a: Inhibitory Treatment.

A non smoker is diagnosed with lung cancer. They are immediately administered a 1750 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 10 days (or other suitable time period) for the rest of their life. Every few months the trichothecene administration may be suspended for 2 weeks (or other suitable time period), the drug vacation insuring any required normal cell cycling may take place, before once again resuming the inhibitory regimen.

Example 1b: Inhibitory Treatment.

A smoker that refuses to quit smoking is diagnosed with lung cancer. They are immediately given a cigarette(s) to smoke capable of delivering a 1750 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene). This administration method is repeated once every 10 days (or other suitable time period) for the rest of their life. Periodic, but brief, drug vacations described in 1a may also be included as part of such regimen.

Example 1c: Cytotoxic Treatment.

A non smoker is diagnosed with lung cancer. They are immediately administered a 3456 ng cytotoxic dose (or any other suitable cytotoxic dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 20 days (or other suitable time period) until no tumor is detected.

Example 1d: Cytotoxic Treatment.

A smoker is diagnosed with lung cancer. They are immediately given a cigarette(s) to smoke capable of delivering a 3456 ng cytotoxic dose (or any other suitable cytotoxic dose), of satratoxin H (or any other suitable trichothecene). This administration is repeated once every 20 days (or

other suitable time period) until no tumor is detected.

Example 2: Use of Therapeutics as Stand Alone Treatment of Breast Cancer in the Lung

Nearly half of patients treated for apparently localized breast cancer develop metastatic disease. Approximately 66% of breast metastasis involve the lungs.

Example 2a: Inhibitory Treatment.

Several months after treatment for breast cancer it is detected that the breast cancer has metastasized into the lungs. The patient is immediately administered a 1750 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 10 days (or other suitable time period) until a determination is made on what chemotherapeutic, surgical, or other procedure will be undertaken to combat the metastasis.

Example 2b: Cytotoxic Treatment.

Several months after treatment for breast cancer it is detected that the breast cancer has metastasized into the lungs. The patient is immediately administered a 3456 ng cytotoxic dose (or any other suitable cytotoxic dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 20 days (or other suitable time period) until no tumor is detected.

Example 3 : Method of Use in Conjunction With Conventional Chemotherapy - The chosen course of treatment for a patient with lung cancer is administration of paclitaxel and carboplatin, the leading cytotoxic chemotherapy for treatment of lung cancer. Paclitaxel and carboplatin are administered intravenously in 3 week cycles.

Example 3a: Insuring Integrity of Skipper Log Cell Kill Model

Immediately after completion of administration of paclitaxel and carboplatin (or any other chemotherapeutic regimen), the patient is administered a 1750 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder

inhaler (or any other suitable method of inhalation). This administration is repeated, once only, in 10 days (or other suitable time period). Administration is suspended until after administration of the next cycle of paclitaxel and carboplatin at which time it is once again administered in the same manner as described above.

Example 3b: MDR Inhibition

A lung neoplasm(s) is unresponsive to treatment with paclitaxel and carboplatin (or any other chemotherapeutic regimen). It is determined the tumor produces large amounts of MDR (multidrug resistance) proteins. The patient is immediately administered a 3456 ng cytotoxic dose (or any other suitable cytotoxic or inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). In 10 days (or other suitable time period) paclitaxel and carboplatin (or any other chemotherapeutic regimen) are once again administered. In 10 days the trichothecenes are once again administered. In 10 days the paclitaxel and carboplatin are once again administered. This "leapfrogging" of trichothecene and prior art chemotherapeutics is repeated for the duration of the 6 or 8 cycles, as prescribed under the prior art chemotherapeutic administration regimen.

Example 3c: Combination Chemotherapy

The chosen course of treatment for a patient with lung cancer is administration of paclitaxel and carboplatin (or any other prior art chemotherapeutic). The patient wants to increase their odds of survival. The patient is immediately administered a 3456 ng cytotoxic dose (or any other suitable cytotoxic dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). In 10 days (or other suitable time period) paclitaxel and carboplatin (or any other chemotherapeutic regimen) are once again administered. In 10 days (or other suitable time period) the trichothecenes are once again administered. In 10 days (or other suitable time period) the paclitaxel and carboplatin (or any other chemotherapeutic regimen) are once again administered. This "leapfrogging" of trichothecene and prior art chemotherapeutics is repeated for the duration of the 6 or 8 cycles, as prescribed under the prior art chemotherapeutic administration regimen.

Example 4: Prophylactic Activity for Patients at High Risk for Lung Cancer

A heavy lifelong smoker, that cannot or chooses not to quit smoking, is approaching 50 years of age, and is at high risk for contracting lung cancer and wants to insure a cancer will not start growing and metastasize before their next examination for lung cancer. The patient is put on a low inhibitory dose administration regimen, comprising smoking a cigarette(s) capable of delivering a 830 ng inhibitory dose (or lower dose), of satratoxin H (or any other suitable trichothecene). This administration method is repeated once every 10 days (or other suitable time period) for a period of 2 months (or other suitable time period) followed by a 2 week drug "vacation" (or other suitable time period) followed again by the resumption of the cyclical 2 months "on" , 2 weeks "off" until roughly 2 weeks before the lung cancer examination. After the examination by PET scan or any other suitable means is performed, the patient may go back on the prophylactic treatment regimen as described above.

Example 5: Palliative Activity for Patients Dying From Lung Cancer

A patient with lung cancer that has metastasized throughout the body is diagnosed as terminal and no curative attempts are undertaken. The prognosis is that within 2 months the patient will die from cancer related complications in the lung, and in the absence of that, in 6 months from other metastasis. The patient desires to have as much time to live to spend the little time they have left with their loved ones The patient is immediately administered a 3456 ng cytotoxic dose (or any other suitable cytotoxic or inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 15 days (or other suitable time period) for the rest of their remaining life.

Example 6: Inhaled Chemical Debridement Method for COPD

A lifelong smoker has severe emphysema and would like some degree of lung function restored however they cannot afford an LVRS or are scared to by the 18% mortality rate. Under present invention they would now have a chemexfoliation / chemical debridement option instead of surgery. The patient is administered a single normal tissue toxic level dose of more than 3800 ng (or any other suitable toxic, but not lethal, dose necessary to kill a desired percentage of non cycling lung cells), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or

any other suitable method of inhalation). The patient is monitored for potential anaphylactic reaction which may be managed by any of several therapies available in under prior art or the more broad spectrum inflammatory inhibition of present invention. The patient is also fitted with a mask to filter any airborne contaminants while the lungs regenerate new tissue in place of the old destroyed cells. Subsequent low inhibitory doses of trichothecenes may also be considered to manage either over proliferation of replacement cells or continued inflammatory suppression.

Example 7: Inhaled, Localized, Suppression of Inflammation or Anaphylaxis

Example 7a: Inhibition of COPD Related Inflammation

A COPD patient that has been a lifelong smoker is having trouble breathing because of ongoing inflammation in the lungs coupled with their destroyed cilia and virtually non existent cephalad movement, which is causing excessive fluid accumulation in the lungs. The patient is started on an administration regimen comprising a 1348 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 10 days (or other suitable time period) for the rest of their life. Every few months the trichothecene administration may be suspended for 2 weeks (or other suitable time period), the drug vacation insuring any required normal cell cycling may take place, before once again resuming the inhibitory regimen.

Example 7b: Allergic Reaction or Allergic Asthma Prevention

A person is going of a trip that will involve exposure to antigens known to trigger a severe allergic reaction or allergic asthma. The patient is given a 1750 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation) a day prior (or other suitable time period) to their expected contact with allergen. This administration may be used in conjunction with prior art drug treatments.

Other Applications and Embodiments

Although the preferred embodiment of present invention focuses on use of therapeutics for treatment of the above disorders, nothing is intended to preclude the use of current invention for

other conditions or disorders of the lungs that could benefit from hyperactive protein synthesis inhibition or inhibition of growth or cytotoxicity to rapidly proliferating cells in the lung. The above examples may include or exclude varying "drug vacation" periods for normal cell replacement to occur. The present invention also envisions mixing the trichothecene with other compounds or substances, including combinations of trichothecenes, to facilitate administration, facilitate or regulate the rate and/or depth of penetration and/or absorption of said trichothecene mycotoxins, increase efficacy of said mycotoxins, provide prophylactic activity against infection, or provide any other beneficial or synergistic function. The compounds collectively described above are termed herein "pharmaceutical compositions". As an example, a combination of macrocyclic and simple trichothecenes may be used to achieve more extensive penetration (as macrocyclics internalize faster and simple trichothecenes would migrate further before internalization). As another example, antibiotics may be included as part of the "pharmaceutical composition" . As another example pharmaceutical compositions may include other protein synthesis inhibitors or angiogenesis inhibitors such as squalamine or troponin. As another example pharmaceutical composition may include any inert ingredients to facilitate or enhance distribution of therapeutics by inhalation. The examples provided in the application are only some of the potential uses of therapeutics of present invention and nothing in this application is intended to limit the potential uses of therapeutics of present invention for treatment of any conditions of the lungs that could benefit from localized inhibition of protein synthesis.

The compositions of present invention cannot be administered to infants and young children. Care would also need to be taken that patients undergoing treatment with therapeutics of present invention do not inadvertently expose infants or young children to therapeutics of present invention.

Related U.S. Application Data
Continuation-in-part of application No. 09/132,153 filed 08/11/98, abandoned.

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